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Hi

I need the following papers to examine 09/234,290, this is a RUSH since this case is due this biweek.

1. Yoon et al, Annals of the NY Academy of Sciences, 2001, 928:200-211

2. Poulton et al, Diabetes/Metabolism Research and Reviews, 2001, 17(6)429-435 *****

3.Hanninen et al, Immunological Reviews, 2000, 173:109-119

4. Green et al (Immunological Reviews, 1999, 169:11-22

5. Simone et al, Diabetes Care, 1999,22 Suppl 2 B7-B15

6. Palmer, J. Clin. Investigation, 2001, 108(1)31-33

7. Seddon et al (Biochem Soc. Transactions,1997, 25(2)620-624)

8. Reddy et al, Histochemical Journal, 2000, 32(4)195-206

9. Ylinen et al, Pancrea, 2000, 20(2)197-205

10. Sainio et al, Pancrea, 1999, 18(3)282-293

11. Alamunits et al, Clinical and Experimental Immunology, 1999, 115(2)260-267.

I also need an entire volume, Cohen et al (Autoimmune Disease Models, A Guidebook, Academic Press, San Diego, 1994

Thanks
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Cellular and Molecular Pathogenic Mechanisms of Insulin-Dependent Diabetes Mellitus

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ABSTRACT: **Insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes, is an organ-specific autoimmune disease resulting from the destruction of insulin-producing pancreatic β cells. The hypothesis that IDDM is an autoimmune disease has been considerably strengthened by the study of animal models such as the BioBreeding (BB) rat and the nonobese diabetic (NOD) mouse, both of which spontaneously develop a diabetic syndrome similar to human IDDM. β cell autoantigens, macrophages, dendritic cells, B lymphocytes, and T cells have been shown to be involved in the pathogenesis of autoimmune diabetes. Among the β cell autoantigens identified, glutamic acid decarboxylase (GAD) has been extensively studied and is the best characterized. β cell-specific suppression of GAD expression in NOD mice results in the prevention of IDDM. Macrophages and/or dendritic cells are the first cell types to infiltrate the pancreatic islets. Macrophages play an essential role in the development and activation of β cell-cytotoxic T cells. B lymphocytes play a role as antigen-presenting cells, and T cells have been shown to play a critical role as final effectors that kill β cells. Cytokines secreted by immunocytes, including macrophages and T cells, may regulate the direction of the immune response toward Th1 or Th2 as well as cytotoxic effector cell or suppressor cell dominance. β cells are destroyed by apoptosis through Fas-Fas ligand and TNF-TNF receptor interactions and by granzymes and perforin released from cytotoxic effector T cells. Therefore, the activated macrophages and T cells, and cytokines secreted from these immunocytes, act synergistically to destroy β cells, resulting in the development of autoimmune IDDM.**

KEYWORDS: **Autoimmune diseases; Diabetes mellitus, insulin-dependent; IDDM; Insulin-dependent diabetes mellitus;**

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INTRODUCTION

The development of insulin-dependent diabetes mellitus (IDDM) results from the destruction of pancreatic β cells by a complicated and chronic pathogenic process of islet-specific autoimmune reactions. Studies on β-cell–specific autoimmunity using two animal models of human IDDM, the nonobese diabetic (NOD) mouse and the BioBreeding (BB) rat, have greatly enhanced our understanding of the pathogenesis of autoimmune diabetes.[1] Cumulative evidence indicates that β–cell autoantigens, macrophages/dendritic cells, B lymphocytes, and T lymphocytes are clearly involved in the complicated pathogenic process of this disease. In this review, we discuss the role of β-cell autoantigens and these immune cells in the pathogenesis of autoimmune IDDM, particularly the results obtained from studies on NOD mice.

ROLE OF β-CELL AUTOANTIGENS

β-cell autoantigens, which are the targets of autoimmune attack in IDDM, have proven difficult to identify. The specificity of circulating autoantibodies present in the sera of IDDM patients and diabetic animals has been investigated extensively. Over 20 years ago, Bottazzo *et al.*[2] and MacCuish *et al.*[3] first detected antibodies directed against the pancreatic islets. Since that time, many studies have revealed that islet cell antibodies are prevalent in patients with IDDM. It is known that peripheral $CD4^+$ T cells from prediabetic and early diabetic patients proliferate in response to islet autoantigens, which react with IDDM-associated autoantibodies. Autoantigens identified in humans, NOD mice, and BB rats include islet cell autoantigens, thought to possess the properties of sialic acid containing glycolipid; insulin; the insulin receptor; a 52-kD protein; a 69-kD protein, glutamic acid decarboxylase (GAD); IA-2, 37/40kD tryptic fragments of a 64-kD antigen (different from GAD); heat shock protein 65 (HSP65); carboxypeptidase H (CPH); the glucose transporter; and a 38-kD autoantigen.[4] The precise role that these autoantigens play in IDDM is not fully understood. Many different approaches have been attempted in order to study the role of β-cell autoantigens, particularly GAD and insulin, which are considered to be the most important autoantigens in IDDM.

GAD. It is believed that GAD is a major islet cell autoantigen; thus, GAD has been extensively studied. In 1990 Baekkeskov *et al.*[5] identified this 64-kD antigen in the pancreatic β cells of IDDM patients as glutamic acid decarboxylase, the biosynthetic enzyme of the inhibitory neurotransmitter gamma-amino-butyric acid (GABA). GAD is mainly localized to synaptic-like microvesicles in β cells. In addition to its presence in β cells, GAD is expressed in the testes, ovaries, thymus, stomach, and brain of mammals as well as in human pancreatic α, δ, and polypeptide producing (pp) cells. However, the role played by GAD in human IDDM remains unknown. To date, two distinct forms of GAD, GAD67 and GAD65, have been identified. These two forms of GAD are encoded by two different genes. The amino acid sequences of GAD67 and GAD65 are approximately 70% homologous. There is a strong variation in the expression of the two isoforms of GAD in the pancreatic islets depending on the species of animal examined.[6,7] Both human and rat islets predominantly express GAD65, whereas GAD67 is predominantly expressed in mouse islets.[7]

Immunization of NOD mice with purified GAD results in the tolerization of GAD-reactive T cells and blocks the development of T-cell responses to other β cell antigens, thus preventing insulitis and diabetes.[8,9] In their 1993 study, Kaufman *et al.*[8] stated that the initial immune response directed against pancreatic islets in NOD mice was a Th1 response to a confined region of GAD (amino acids 509-528 and 524-543), and later responses were directed against another region of GAD (amino acids 246-266) and other autoantigens, such as HSP65 and insulin. Although no GAD-reactive $CD8^+$ T cells have been isolated from NOD mice, GAD-reactive $CD4^+$ Th1 cells isolated from diabetic NOD mice induced diabetes in NOD.severe combined immunodeficiency disease (*scid*) mice.[10] These results suggest that GAD plays an important role in the pathogenesis of autoimmune diabetes. However, controversy surrounds the role played by GAD in the pathogenesis of IDDM. Chen *et al.*[11] have studied the reactivity of T cells to a GAD65-derived peptide, GAD65 residue 524-543, in NOD mice and two congenic NOD strains, B10.H-2^{g7} and NOD.B$6^{I12\text{-}Tshb}$. They demonstrated that the response to GAD65 524-543 was MHC class II-restricted and that T-cell responses to GAD-derived peptides can be elicited in mice resistant to the development of spontaneous IDDM. Thus, Chen *et al.* suggested that peripheral tolerance to GAD is not associated with the prevention of diabetes. To further investigate the role of GAD in the pathogenesis of IDDM, transgenic strategies have been used. The overall expression of GAD, after the cloning of GAD65 under the MHC class I promoter in NOD mice, accelerated the onset and increased the incidence of the disease.[12] In addition, transgenic NOD mice that express GAD65 in the β cells were established. One line, which showed high expression of GAD65, showed a preventive effect on diabetes, but another line showed no difference from control NOD mice.[13] Therefore, the role of GAD remains uncertain. To determine the role of GAD, we selectively suppressed GAD expression in the β cells of diabetes-prone NOD mice and observed whether this resulted in the prevention of autoimmune IDDM. Our recent study showed that β cell-specific suppression of GAD expression in two lines of antisense GAD transgenic mice resulted in the prevention of autoimmune diabetes, whereas any level of GAD expression in the β cells in other lines of antisense GAD transgenic NOD mice resulted in the development of autoimmune diabetes, similar to that seen in transgene-negative NOD mice.[14] These results indicate that GAD may be a triggering autoantigen in the development of autoimmune IDDM in NOD mice.

Insulin. Insulin is a logical candidate for an autoantigen of IDDM, because insulin is the only known β-cell–specific antigen related to IDDM. It has been reported that the oral intake of insulin retards disease progression in the NOD mouse as a result of the induction of immunoregulatory T cells.[15] In addition, the intrathymic injection or the subcutaneous or intranasal administration of the insulin B chain in NOD mice prevents diabetes.[16] Metabolically inactive insulin obtained by changing one amino acid in the B chain also has a preventive effect.[16] Insulin B chain-specific $CD4^+$ T cell clones identified in NOD mice accelerate diabetes in young NOD mice and adoptively transfer the disease in NOD.*scid* mice.[17] Regulatory T cells reactive to insulin have been isolated and shown to have a preventive effect in NOD mice. More recently, a diabetogenic $CD8^+$ T cell clone, which causes diabetes in neonatal NOD mice, was found to recognize insulin B chain amino acids 15-23.[18] These results indicate that insulin plays an important role as an autoantigen in IDDM in NOD

mice. Anti-insulin antibodies (IAAs) have been detected in more than 59% of patients diagnosed with late preclinical/recent onset IDDM. However, the pathogenic role of IAAs and insulin-reactive T cells needs further investigation. There is an interesting report that examines cross-reactivity between insulin and the islet-expressed retroviral antigen p73.[19] However, the role of this cross-reactivity in the pathogenesis of autoimmune IDDM is not known.

38-kD Antigen. Anti-38-kD autoantibodies were originally identified in human diabetic sera. Roep *et al.*[20] identified a 38-kD antigen, which was recognized by a T-cell clone established from newly diagnosed IDDM patients, from the insulin secretory granule. Recently, these researchers cloned and sequenced a novel murine cDNA encoding this antigen, named imogen 38.[20] We found that the 38-kD antigen in BB rats is the only delayed-expressed islet cell autoantigen whose antibody is consistently found in acutely diabetic DP-BB rats.[21] As a result of its delayed expression, this 38-kD autoantigen may be considered 'nonself,' which may trigger β cell-specific autoimmunity. Whether there are any molecular similarities between imogen 38 and our delayed-expressed 38-kD islet cell autoantigen remains to be determined. Interestingly, we found that CMV induces antibodies directed against the 38-kD antigen in humans[22] but in this instance the role of the 38-kD autoantigen remains to be determined.

IA-2 Autoantigen (37/40kD Tryptic Fragment). IA-2 is a newly discovered member of the protein tyrosine phosphatase (PTP) family and is considered to be one of the major autoantigens of IDDM. The IA-2 protein is the precursor to the 37 and/or 40-kD islet tryptic fragment.[23] Autoantibodies directed against IA-2 have been detected in 70% of IDDM patients. But these autoantibodies are not detected in NOD mice or BB rats. The IA-2 autoantigen from a rat β-cell line (RIN5AH) reacts with sera from IDDM patients.[23] Antibodies to the IA-2 autoantigen, but not anti-GAD antibodies, react with ICAs in patients that rapidly developed IDDM.[24] However, the precise role of the IA-2 antigen in the pathogenesis of IDDM is unknown.

ROLE OF MACROPHAGES

The major populations of cells infiltrating the islets during the early stage of insulitis in BB rats and NOD mice have been shown to be macrophages and dendritic cells.[25] This infiltration precedes invasion of the islets by T lymphocytes, natural killer (NK) cells, and B lymphocytes. In addition, electron microscopy has revealed that most of the single cells present at an early stage of insulitis in BB rats are macrophages.[26] The inactivation of macrophages in NOD mice and BB rats with silica, a substance that is toxic to macrophages, results in the near complete prevention of insulitis and diabetes. This result suggests that macrophages play an important role in the development of insulitis and diabetes in these animal models. However, the precise role of macrophages in T-cell–mediated autoimmune diabetes remains unknown.

We first examined whether macrophages are required for the development of the effector T cells that destroy β cells. Splenocytes from macrophage-depleted NOD mice by liposomal dichloromethylene diphosphonate (lip-Cl_2MDP) did not transfer diabetes to NOD.*scid* mice, whereas those from control NOD mice in which mac-

rophages were present did, indicating that macrophages are required for the development of β-cell–cytotoxic effector T cells in NOD mice. Our further study showed that T cells in the macrophage-depleted NOD recipients did not destroy the transplanted NOD islets, indicating that T cells in a macrophage-depleted environment lose their ability to differentiate into cytotoxic T cells that can destroy pancreatic β cells.[27] However, these T cells regained their β-cell cytotoxic potential when returned to a macrophage-containing environment.

To learn why T cells in a macrophage-depleted environment lose their ability to kill β cells, we examined the islet antigen-specific immune response and T-cell activation in macrophage-depleted NOD mice. There was a shift in the immune balance, a decrease in the Th1 immune response, and an increase in the Th2 immune response due to the reduced expression of the macrophage-derived cytokine interleukin (IL)-12. Moreover, there was a deficit in T-cell activation evidenced by significant decreases in the expression of Fas ligand and perforin. The administration of IL-12 substantially reversed the prevention of diabetes in NOD mice conferred by macrophage depletion.[27] We conclude that macrophages play an essential role in the development and activation of β-cell–cytotoxic T cells that cause β-cell destruction, resulting in autoimmune diabetes in NOD mice.

In addition, we investigated the role of macrophages in the development and activation of β-cell cytotoxic $CD8^+$ T cells in T cell-receptor (TCR)-transgenic NOD mice by the adoptive transfer of splenic T cells from macrophage-depleted TCR-β transgenic NOD mice into NOD.*scid* mice.[28] We found that none of the NOD.*scid* recipients developed diabetes up to 10 weeks after transfer, whereas most of the NOD.*scid* recipients of splenic T cells from age-matched control TCR-β transgenic NOD mice became diabetic. When intact NOD islets were transplanted under the renal capsule of macrophage-depleted 8.3-TCR-β transgenic NOD mice, most of the grafted islets remained intact, whereas most of the islets grafted into age-matched, control 8.3-TCR-β transgenic NOD mice were destroyed within 3 weeks after transplantation. The depletion of macrophages in these mice resulted in a decrease in the Th1 immune response along with an increase in the Th2 immune response and a decrease in β-cell–specific T-cell activation, as shown by significant decreases in the expression of FasL, CD40 ligand (CD40L), and perforin, as compared with control mice. As shown in NOD mice, macrophages are absolutely required for the development and activation of β-cell–cytotoxic $CD8^+$ T cells that cause β–cell destruction, which leads to diabetes in 8.3-TCR-β transgenic NOD mice.[28]

Although further studies to elucidate the precise mechanism of the involvement of macrophages in T-cell activation remain to be performed, our studies have shown that IL-12 secreted by macrophages may activate Th1-type $CD4^+$ T cells, and subsequently, the IL-2 and interferon (IFN)-γ produced by these activated $CD4^+$ T cells may assist in maximizing the activation of $CD8^+$ T cells. The downregulation of islet cell-specific T-cell activation may be another major factor contributing to the impairment of the capability of T cells to kill β cells in macrophage-depleted NOD mice.

In addition to the role of macrophages in the T-cell–mediated destruction of β cells, we also examined other factors that may be involved in the destruction of these cells. These include macrophage-derived soluble mediators such as oxygen-free radicals and other cytokines including IL-1β, tumor necrosis factor (TNF)-α, and IFN-γ. We found that expression of cytokines IL-1β, TNF-α, and IFN-γ was significantly

decreased in macrophage-depleted NOD mice as compared with PBS-treated control NOD mice. These cytokines, which are released from activated macrophages, are believed to be toxic to β cells.[29,30] The toxic effect produced by activated macrophages on β cells is thought to be mediated by the superoxide anion and hydrogen peroxide. The β cell is very sensitive to the production of free radicals because islet cells exhibit very low free radical scavenging activity. Cytokines produced by islet-infiltrating macrophages may contribute to β–cell damage by inducing the production of oxygen-free radicals in the islets.[31]

ROLE OF B CELLS

Converging data suggest that B cells play a critical role as antigen-presenting cells (APCs) of β-cell autoantigens in the pathogenesis of autoimmune diabetes in NOD mice. Previously, the function of B cells was analyzed as the production of autoantibodies against β-cell autoantigens, which is considered to be a secondary phenomenon of β-cell destruction. T lymphocytes from diabetic NOD mice transfer diabetes to neonatal recipients in the absence of B cells, indicating that B cells are not required for the destruction of β cells after diabetogenic effector T cells are generated. However, later studies demonstrated that B cells are critical APCs for the initiation of T-cell–mediated autoimmune diabetes in NOD mice. B-cell–deficient NOD mice did not develop diabetes,[32] and the depletion of B cells by anti-*u* antibody treatment completely abrogated the development of insulitis.[33] More recently, it was reported that B-cell–specific I-A^{g7}-deficient NOD mice showed peri-insulitis, but converted to destructive insulitis after cyclophosphamide (CY) treatment. This result suggests that I-A^{g7}-mediated β–cell autoantigen presentation by B cells is critical in overcoming a checkpoint in T-cell tolerance to pancreatic β cells after their initial targeting has occurred.[34]

ROLE OF T CELLS

Substantial evidence from studies using BB rats and NOD mice supports a critical role of T cells in the pathogenesis of autoimmune type I diabetes. It has been shown that the development of diabetes was prevented by neonatal thymectomy in BB rats,[35] and BB rats treated with monoclonal antibodies (OX-19) directed against the antigens expressed on the surface of all T cells do not develop diabetes, indicating that T cells play an important role in the destruction of β cells.[36] In addition, lymphocytes from diabetic BB rats transfer the disease to young diabetes-prone BB rats.

In the NOD mouse model, it is clear that T cells play a critical role in the development of autoimmune diabetes. Athymic NOD mice and NOD.*scid* mice do not develop insulitis or diabetes.[37,38] Treatment of NOD mice with anti-CD3 antibodies inhibits the development of diabetes.[38] In addition, most transfer studies of NOD splenic T cells into NOD mice show that the transfer of diabetes requires both $CD4^+$ and $CD8^+$ T cells.[40,41] Islet cell-specific T-cell clones have been isolated from insulitic lesions and the splenocytes of both prediabetic and diabetic NOD mice. Some $CD4^+$ islet-specific T-cell clones[42] accelerate the development of diabetes in young

NOD mice and destroy islet grafts in $CD8^+$ T-cell–depleted diabetes-resistant mice. The BDC2.5/NOD transgenic mouse, which expresses the rearranged T-cell receptor (TCR) α and β chain genes of this $CD4^+$ T cell clone (BDC2.5),[42] exhibits an increased incidence of diabetes.[43]

In addition to $CD4^+$ T cell involvement in β–cell destruction, experimental evidence reveals that $CD8^+$ T cells play a role as effector cells in the destruction of β cells in the NOD mouse. $CD8^+$ cytotoxic T cell clones (CTLs), isolated from the islets of diabetic NOD mice, destroy β cells *in vitro* and transfer diabetes *in vivo* with the help of $CD4^+$ T cells.[44] Some $CD8^+$ T cell clones have been shown to destroy β cells without the help of $CD4^+$ T cells.[45] We have cloned a dozen islet-reactive $CD4^+$ and $CD8^+$ T cells from the lymphocytes infiltrating the pancreatic islets of NOD mice.[44] All $CD4^+$ T cells are restricted to MHC class II of NOD, I-A^{g7}. One of our $CD4^+$ T cell clones (NY4.1) responded only to islet cells from NOD mice, indicating that the NY4.1 clones recognize the islet antigen with a unique I-A^{nod} molecule on the islet cell or the intrinsic APCs. The remaining $CD4^+$ T cell clones showed a proliferative response to both islet cells and spleen cells from NOD mice, but not from other strains of mice including SJL, C3H, C57BL/6, and DBA/2 mice, indicating that these clones are I-A^{g7} reactive T cells (autoreactive T cells). None of these $CD4^+$ T cell clones, either islet-specific or autoreactive, had any cytotoxic effect on NOD islet cells *in vitro*.[44] In contrast, the $CD8^+$ T cell clones did exhibit cytotoxic activity to NOD islets *in vitro*. Furthermore, the proliferative response and cytotoxic activity of some $CD8^+$ T-cell clones was blocked almost completely by anti-MHC class I D^b monoclonal antibodies and that of other $CD8^+$ T cell clones was blocked by anti-MHC class I K^d monoclonal antibodies. These results indicate that $CD8^+$ T cell clones respond to islet cells with the restriction of MHC class I. Electron microscopic studies revealed that islet-specific $CD4^+$ T cells attached closely to islet cells but did not destroy them *in vitro*. In contrast, all of the $CD8^+$ T-cell clones showed a cytotoxic effect on the islet cells and the $CD8^+$ T cells showed pseudopod-like protrusions into the β cells, but not into α or δ cells, leading to the selective destruction of β cells *in vitro*.[44] These results suggest that $CD4^+$ and $CD8^+$ T cells interact differently with β cells during T-cell–mediated β–cell destruction. $CD4^+$ T cells secrete cytokines including IL-2 and IFN-γ, which in turn help maximally activate $CD8^+$ T cells. $CD8^+$ T cells may act as final effector cells directly involved in β–cell destruction. As a matter of fact, $CD8^+$ T cells produce cytokines that may upregulate Fas within the islet, and the destruction of β cells could take place both specifically in relation to the production of perforin as well as nonspecifically through the Fas/FasL interaction. However, both $CD4^+$ and $CD8^+$ T cells, by producing inflammatory cytokines including TNF and IFN-γ, can upregulate Fas on the islets. Once Fas is upregulated on the islets, FasL-expressing $CD4^+$ Th1 cells and $CD8^+$ T cells may induce apoptosis to kill β cells (FIG. 1).

Among our $CD4^+$ and $CD8^+$ T-cell clones, an H-2^{g7}-restricted $CD4^+$ T cell clone, NY4.1, and an H-$2K^d$-restricted $CD8^+$ T-cell clone, NY8.3, were chosen for further study. TCR transgenic NOD mice expressing the rearranged TCR-α and/or -β genes derived from the diabetogenic $CD8^+$ T-cell clone[44] were established. The TCR-β transgenic NOD mice showed a 10-fold increase in the peripheral precursor frequency of β-cell–specific $CD8^+$ T cells and a selective acceleration of the recruitment of $CD8^+$ T cells to the pancreatic islets of prediabetic NOD mice.[46] The TCR-αβ trans-

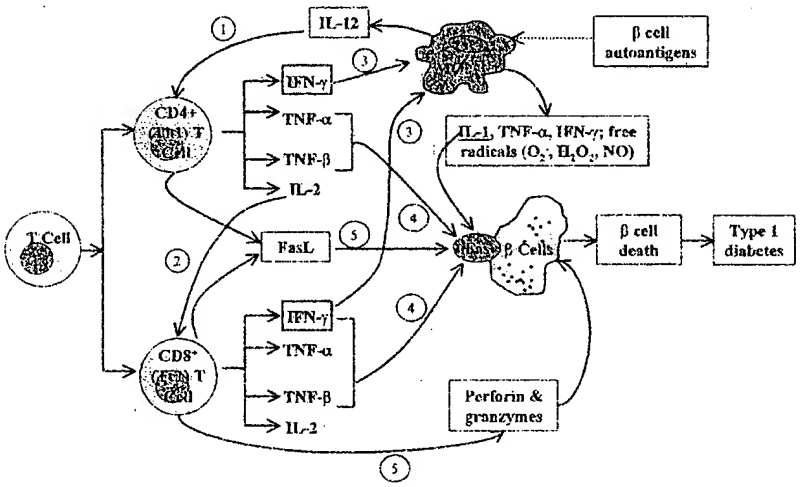

FIGURE 1. Schematic representation of the collaboration between macrophages and T cells in the destruction of pancreatic β cells. β–cell autoantigens may be released from the β cells during spontaneous turnover of β cells. The antigens are then processed by macrophages and presented to helper T cells in conjunction with MHC class II molecules. The activated macrophages may secrete IL-12, which activates Th1 type $CD4^+$ T cells (**1**). The $CD4^+$ T cells secrete cytokines such as IFN-γ, TNF-α, TNF-β and IL-2. While this process is taking place, β–cell–specific precytotoxic T cells may be recruited to the islets. These precytotoxic T cells may be activated by IL-2 and other cytokines released by $CD4^+$ helper T cells to differentiate into $CD8^+$ effector T cells (**2**). IFN-γ released by helper T cells and cytotoxic T cells may cause macrophages to become cytotoxic (**3**). These cytotoxic macrophages release substantial amounts of β cell-toxic cytokines (including IL-1β, TNF-α, and IFN-γ) and free radicals (H_2O_2, NO). Cytokines released from macrophages and T cells may induce the expression of Fas on pancreatic β cells (**4**). β cells are destroyed by Fas-mediated apoptosis (**4**) and/or granzyme and cytolysin (perforin), which are toxic to β cells (**5**).

genic NOD (8.3-NOD) mice showed a 400-fold increase in the peripheral precursor frequency of β-cell–specific $CD8^+$ T cells and a selective acceleration of the recruitment of $CD8^+$ T cells to the pancreatic islets of prediabetic NOD mice.[47] These mice showed an earlier onset and more rapid progression of β–cell destruction, resulting in acceleration of the onset of diabetes as compared with that in nontransgenic NOD mice. In addition, TCR transgenic NOD mice expressing the T-cell receptor (TCR)α and TCRβ chains of the $CD4^+$ T cell clone, NY4.1, showed an accelerated onset of diabetes.[47]

Cytokines produced by T cells also play an important role in the pathogenesis of autoimmune type 1 diabetes.[48] In general, Th1 cytokines (IL-2, IFN-γ) have been shown to be involved in the development of the disease, while Th2 or Th3 cytokines (IL-4, IL-10, TGF-β) have been involved in the prevention of the disease. However, the role of cytokines in the pathogenesis of autoimmune type 1 diabetes is complex. For example, the treatment of NOD mice with anti-IFN-γ prevents the development of diabetes and the transgene expression of IFN-γ in the β cells of normal mice results in the development of type 1 diabetes. However, the genetic absence of IFN-γ

in NOD mice results in a delay in the development of diabetes, but does not prevent it. The systemic administration of IL-4 or IL-10 prevents type 1 diabetes in NOD mice and the transgenic expression of IL-4 on β cells prevents the development of diabetes. However, the local expression of IL-10 in the islets accelerates the development of diabetes in NOD mice, and IL-4 knockout NOD mice did not show accelerated disease onset. Therefore, the interactions of the many different cytokines in the immune system are complicated and the development of diabetes may depend upon which way the finely tuned balance of immunoregulatory cytokines is tipped.

Recently, possible mechanisms for T-cell–mediated β–cell destruction have been elucidated. $CD8^+$ cytotoxic T cells destroy β cells through the perforin and granzyme pathway as well as through the Fas/Fas-L interaction. NOD mice lacking perforin expression were found to develop insulitis, but not diabetes.[49] Fas-deficient NOD mice were found to be free of diabetes and insulitis, and Fas-mediated apoptosis of the β cells was suggested to be the major mechanism for β–cell damage.[50,51] On the other hand, TNFα/TNFα-receptor-mediated apoptosis may also play a greater role in the destruction of β cells by $CD4^+$ T cells.[52]

CONCLUSION

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease with a multifactorial etiology. Thus, a clear understanding of the pathogenic mechanisms involved in the etiology of this disease is not easy. Animal models such as nonobese diabetic (NOD) mice and BioBreeding (BB) rats, which spontaneously develop autoimmune diabetes similar to human autoimmune IDDM, have been used to study the pathogenic mechanisms of autoimmune IDDM. β–cell autoantigens, macrophages/dendritic cells, B cells, and T cells play important roles in the development of the disease in NOD mice. Among the β–cell autoantigens identified, glutamic acid decarboxylase (GAD) and insulin are considered to be the most important autoantigens in the development of IDDM. However, the role of these autoantigens remains to be determined. B cells clearly play a role as antigen-presenting cells, particularly of β–cell autoantigens. Macrophages, which infiltrate the islets at the early stage of insulitis, are considered to be primary contributors to the immune environment conducive for the development and activation of β-cell–cytotoxic T cells. Both $CD4^+$ and $CD8^+$ T cells play a role as final effectors in the destruction of pancreatic β cells. Although the animal models studied do not show a diabetic syndrome identical to human autoimmune IDDM, the information obtained through studies using these animal models will be invaluable for understanding the pathogenic mechanisms of human IDDM and for the development of strategies for the prevention of the disease.

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